

Claims

1. A method of isolating a pyruvate formate-lyase (Pfl) defective lactic acid bacterium, the method comprising the steps of

- (i) providing a wild-type lactic acid bacterial strain which under aerobic conditions is not capable of growth in the absence of acetate in a medium not containing lipoic acid, but which is capable of growth in such medium under anaerobic conditions, and
- (ii) selecting from said wild-type strain a mutant which under said conditions essentially does not grow in the absence of acetate.

2. A method according to claim 1 wherein the wild-type strain prior to the selection of a mutant is subjected to a mutagenization treatment and the thus mutagenized strain is cultivated under anaerobic conditions in the medium not containing lipoic acid in the absence or presence, respectively of acetate and a mutant is selected which under said conditions essentially does not grow in the absence of acetate.

3. A method according to claim 1 wherein the lactic acid bacterium is selected from the group consisting of a *Lactococcus* species, a *Lactobacillus* species, a *Leuconostoc* species, a *Pediococcus* species, a *Streptococcus* species and a *Bifidobacterium* species.

4. A method according to claim 3 wherein the lactic acid bacterium is a *Lactococcus lactis*.

5. A method according to claim 4 wherein the lactic acid bacterium is *Lactococcus lactis* subspecies *lactis*.

6. A Pfl defective mutant lactic acid bacterium which is obtainable by the method of claim 1 and having, relative to the wild-type strain from which it is derived, at least one of the following characteristics:

- (i) essentially the same growth rate when cultivated under aerobic conditions in M17 medium,

- (ii) a reduced growth rate or a reduced rate of acid production when cultivated under anaerobic conditions in M17 medium or in reconstituted skim milk (RSM),
- (iii) essentially no production of formate under the anaerobic conditions of (ii),
- (iv) a reduced production of ethanol or acetate under said above anaerobic conditions, and/or
- (vi) an increased production of at least one α -acetolactate-derived metabolite when cultivated under anaerobic conditions in RSM.

7. A lactic acid bacterium according to claim 6 which has a production of acetoin which is increased by at least 100%.

8. A lactic acid bacterium according to claim 6 which is selected from the group consisting of a *Lactococcus* species, a *Lactobacillus* species, a *Leuconostoc* species, a *Pediococcus* species, a *Streptococcus* species and a *Bifidobacterium* species.

9. A lactic acid bacterium according to claim 8 which is a *Lactococcus lactis*.

10. A lactic acid bacterium according to claim 9 which is selected from the group consisting of *Lactococcus lactis* subspecies *lactis* and *Lactococcus lactis* subspecies *lactis* biovar *diacetylactis*.

11. A lactic acid bacterium according to claim 10 which is selected from the group consisting of *Lactococcus lactis* subspecies *lactis* strain DN221 deposited under the accession No. DSM 11034, a strain essentially having the characteristics of DSM 11034, *Lactococcus lactis* subspecies *lactis* biovar *diacetylactis* strain DN227 deposited under the accession No. DSM 11040 and a strain essentially having the characteristics of DSM 11040.

12. A Pfl and Ldh defective mutant lactic acid bacterium which is not capable of growing under anaerobic

conditions in the presence of acetate, said bacterium being obtainable by the method of claim 1 and having, relative to a wild-type lactic acid bacterium or its Pfl defective parent strain, at least one of the following characteristics:

- (i) essentially the same growth yield when cultivated under aerobic conditions in M17 medium,
- (ii) a reduced capability of converting lactose to lactate,
- (iii) an increased production of α -acetolactate, and/or
- (iv) an increased production of an α -acetolactate derived metabolite.

13. A mutant lactic acid bacterium according to claim 12 having an α -acetolactate production which is increased by at least 100%.

14. A mutant lactic acid bacterium according to claim 12 having an α -acetolactate derived metabolite production which is increased by at least 100%.

15. A lactic acid bacterium according to claim 12 which is selected from the group consisting of a *Lactococcus* species, a *Lactobacillus* species, a *Leuconostoc* species, a *Pediococcus* species, a *Streptococcus* species and a *Bifidobacterium* species.

16. A lactic acid bacterium according to claim 13 which is a *Lactococcus lactis*.

17. A lactic acid bacterium according to claim 16 which is *Lactococcus lactis* subspecies *lactis*.

18. A lactic acid bacterium according to claim 12 in which at least 15% of pyruvate being catabolized is converted to acetoin.

19. A lactic acid bacterium according to claim 17 which is *Lactococcus lactis* subspecies *lactis* DN223 deposited under the accession No. DSM 11036 or a strain essentially having the characteristics of DSM 11036.

20. A lactic acid bacterium which is a mutant or variant of the lactic acid bacterium of claim 12, and is capable of growing anaerobically.

21. A mutant or variant according to claim 20 which is Ldh defective and has the wild-type Pfl activity.

22. A mutant or variant according to claim 21 which when grown anaerobically in reconstituted skim milk powder produces in excess of 1 mM acetoin and/or in excess of 10 mM formate.

23. A mutant or variant according to claim 22 which is *Lactococcus lactis* subspecies *lactis* DN224 deposited under the accession No. DSM 11037 or a strain essentially having the characteristics of DSM 11037.

24. A mutant or variant according to claim 20 which is Pfl defective and has the wild-type Ldh activity.

25. A method of producing a food product, the method comprising adding to the food product starting materials a culture of a lactic acid bacterium selected from the group consisting of the lactic acid bacterium of claim 6.

26. A method of producing a lactic acid bacterial metabolite, the method comprising cultivating a lactic acid bacterium according to claim 6 under conditions where the metabolite is produced, and, if required, isolating the metabolite from the culture.

27. A lactic acid bacterial starter culture composition comprising a lactic acid bacterium of claim 6.

28. A lactic acid bacterial starter culture composition comprising a lactic acid bacterium of claim 12.

29. A lactic acid bacterial starter culture composition comprising a lactic acid bacterium of claim 20.